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The American Journal of Medical Technology

MARCH, 1946

Vol. 12, No. 2

OFFICIAL PUBLICATION
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AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS
Published Bi-Monthly by The American Society of Medical Technologists
Printed by The Wines Printing Company
Business Office: Medical Center Bldg., Lafayette, La.
Editorial and Printing Office: 4310 W. Fort St., Detroit 9, Michigan

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  is a highly selective medium for isolation of *Eberthella typhosa*. The unusual selective properties of this medium permit the use of large inocula of feces and other suspected material without overgrowth of extraneous intestinal bacteria.

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VOLUME 12 MARCH, 1946 NUMBER 2

GASTRIC ASPIRATION CULTURE AND THE CONTROL OF TUBERCULOSIS*

By SYLVIA ANDERSON, M.T. (ASCP)

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Tuberculosis control will be made more effective when culturing of gastric aspirations is adopted as routine procedure by every laboratory in which there is a department of bacteriology. The results obtained in tuberculosis hospitals carrying out a consistent culture program on fasting stomach contents of patients indicate that if the practice would be universally adopted by clinics and general hospitals, large and small, it would greatly increase the security of patients, their relatives and visitors, hospital personnel, and the community at large. Plunkett and Mikol[2] estimate that there were probably more than 40,000 unrecognized cases of tuberculosis hospitalized in the United States in 1937, or about 1.2% of the total hospital admissions. Farber and Clark[1] call attention to the fact that "tuberculosis patients commonly suffer from associated pathological conditions for tuberculosis is antagonistic to few disease processes." In a report by the latter of 100 patients admitted to a general hospital with non-tuberculous diseases and later found to have tuberculosis, 81% had far-advanced tuberculosis. The majority were suspected on the basis of symptoms rather than physical

*Award paper (second), June, 1945. (Reprinted by request.)

signs. Some of them had only tuberculosis and did not have the disease recorded at time of admission. On the basis of Plunkett and Mikol's[2] study an additional 300 tuberculous patients probably remained on general wards in the same hospital and were eventually without ever having been diagnosed as tuberculous. Obviously much more careful investigative work could be done if the medical staff were more tuberculosis minded and the hospital laboratories were equipped with the facilities and the skilled technologists.

Culturing of gastric aspirations and/or other suspected material accomplishes two major objectives.

1. Discovery of minimal or symptom-free cases at a stage of development when the prognosis is most favorable;

2. Continuous hospitalization of tuberculosis victims until their disease is no longer infectious.

From a third to a half of sanatorium patients produce little or no sputum. Nobody knows the proportion of unrecognized cases at large who are without sputum. The presence or absence of sputum depends upon the age, sex, mentality, and habits of the individual, and upon the location of the lesion, and on the stage of advancement and degree of activity of the disease. More minimal cases enter the sanatorium during peacetime than during the war. They are hospitalized because of contact with the tuberculous at school, at home or at work; or because of suspicious x-ray pictures or positive tuberculin reactions.

Gastric aspirations from patients, in and out of the hospital, have been cultured extensively at Muirdale Sanatorium since 1939. The results have furnished valuable information for diagnosis and treatment. In the recent literature Steenkin, Yaeger, and Heise[3] conclude: "When tubercle bacilli are difficult to find, persistent and careful bacteriological studies should be made of the sputum and gastric contents, especially by culture and animal inoculations." Robinson and Dunn[4] advise the same measures.

In our hospital the physicians, assisted by the nurses, collect the fasting stomach contents. A small calibre, flexible rubber tube is inserted through the nasal passage and into the stomach, shortly after the patient awakes in the morning. Tubercle bacilli swallowed during sleep are thus recovered before the stomach becomes active. Sometimes a few cubic centimeters of saline or sterile water is

used to facilitate. It is desirable to keep the volume of the specimen under 50 cubic centimeters. The majority of those we examined were from 15 to 20 cubic centimeters. The same 50 cc round bottom pyrex centrifuge tube with constricted neck, fitting size 0-0 rubber stopper, serves for collection and for subsequent preparation for culture.

Technic

Enough KOH solution is mixed with the material to make a concentration of 1½% hydroxide and the mixture is kept at 40° Centigrade for 30 minutes or long enough to become warm. This treatment dissolves the protein of elastic fibers, body cells, and mucus, kills the contaminating bacteria except resistant ones such as tubercle bacilli and spores, and makes a finely divided suspension, suitable for centrifuging.

The liquified specimen is centrifuged at high speed for 20 minutes, decanted and re-suspended in a few cubic centimeters of saline. A drop of brom-cresol-purple solution is added before neutralizing with 5% HCI. By pouring off the fluid before addition of acid, one avoids re-precipitation of protein. Keeping the solids finely divided makes possible a smoother smear and a more evenly distributed culture slant.

The neutral residue is washed and re-centrifuged in about 40 cc of saline, after which it is ready for smear, culture, and/or animal inoculation. Use the entire sediment.

We are staining smears by the Petroff-Schain[5] modification of Ziehl-Neelsen, and find it very economical of time and dyes, especially if 10 or more smears are to be stained. Recently an improved method using propylene glycol as a solvent[6] has come to our attention.

If no organisms are found by smear or if they were seen for the first time on the concentrated smear of any patient, the remainder of the concentrate is divided among three slants of Petragnani's medium (modification used in laboratories of Ontario Department of Health) in tubes 155x27x17 mm. The tubes are incubated in a nearly horizontal position until no moisture is visible on the surface of the slant (usually 48 hours). Then the tubes are sealed a 2:1 paraffin-vaseline mixture to prevent excessive drying of the medium

and the incubation continues for a period up to 8 weeks or until colonies appear. When macroscopic growth appears, smears are always made on any first positive and as often thereafter as common sense dictates to avoid errors in judgment.

A word about smearing and staining colonies is apropos. The slide is warmed, a small drop of water is placed in the middle. A long, pointed inoculating needle is used to fish out a small crumb of growth. The end of the needle is rotated in the droplet of water. As the water evaporates, the particle is rubbed back and forth to make it adhere to the glass.

Culture smears are best stained individually to avoid possible contamination of negative smears in the same batch. Alcoholic solution of carbol fuchsin with a few crystals of pure sodium chloride sprinkled over the dye, is preferable to steaming with a flame.

While no tabulated account was made of the number of colonies appearing on positive cultures, it is safe to say that those having more than 10 were the exceptions and that there were more positive cultures being reported with fewer than five colonies than with more than five. It is common to find only one slant out of three with growth. For every patient with a positive culture, the colony or colonies in question were smeared and stained at least once. Whenever any quality of a growth, as observed grossly or microscopically, appeared to vary from the usual, this colony was tested by sub-culture for ability to grow at room temperature, or ability to grow in the incubator in a shorter period of time than pathogenic acid-fast bacilli require. It was rarely necessary to use guinea pig inoculation to clear up doubts about pathogenicity, but animal inoculation is recommended for the inexperienced worker dealing with doubtful colonies. No instance of mistaken diagnosis due to fallacious positive report has come to our knowledge.

Results of the Cultures

These counts and statistics are based on 3,000 some gastric aspirations made on about 800 Muirdale Sanatorium patients from Jan. 1, 1942, to Jan. 1, 1944. Some patients had only one test, the majority had several.

There were a total of 3,038 smears stained and read, of which

2,891 were negative for tubercle bacilli and 147 were positive. This proportion shows that for gastric aspiration, concentrated smears alone are not fruitful.

The 2,891 sediments having negative smears were cultured, plus another 91 specimens, some of which were without smears because of insufficient material and some of which had positive smears which it was deemed advisable to verify. Accordingly, a total of 2,982 cultures were harvested. Of this number, 2,163 gave negative cultures, 783 were positive, and 36 were failures because of contamination. It should be remembered that tuberculous individuals do not have consistently positive gastric cultures, especially while their condition shows improvement. Since Jan., 1944, we have put into practice a system of pooling a series of consecutive gastric aspirations from the same patient. The majority have five tests done and these are treated, smeared, and cultured as one specimen. It will be interesting to observe to what extent the pooling procedure will change the ratio of positive and negative cultures.

Of the total number of 783 positive cultures mentioned above, 68 were associated with positive smear and 715 had shown negative smear or no smear before culturing.

On the other hand, of the total number of 2,163 negative cultures obtained, 2,141 were derived from specimens with negative smears and 22 from specimens whose smears had acid-fast bacilli. One can assume that the 22 discrepancies occurred because some tubercle bacilli are less resistant to hydroxide solution and so failed to survive the treatment.

It is interesting to note that in 1941 the percentage of[7] positive gastric aspirations cultures was only 16.8% as compared with 26.25% at the present time. The percentage of failures through contamination has dropped from 3.3% to 1.206% in the same period. There is no reason to believe that the incidence of tuberculosis among patients aspirated prior to 1941 was less than it is now. Certainly our doctors have learned to make a more intelligent and discriminating selection of the patients who need gastric aspirations. However, a share of the credit for the notable increase in the proportion of positive gastric aspiration cultures rightly belongs to a new and more efficient incubator which was installed in the fall of 1941.

One more study showing the distribution of positive cultures through the incubation period follows. The earliest colonies appear in about 16 days. The largest number are reportable in the 20 to 23-day period. Approximately 89% of the positives have been reported by the end of the fifth week. The average length of time required for positive cultures was 25 days. The accompanying graph shows percentages for each four-day interval, beginning at 16 days. Obviously, then, the average positive culture is reportable in one-half the time a positive guinea pig test requires. The chick embryo method is the fastest of all, if one counts the age of the embryo at the time of inoculation, about 15 days.[8] I have great admiration for the pioneer work which technologists Galligher and Kurachi did with chick embryos as reported in their paper at the 1943 convention. Perhaps the chick embryo method will some day be developed to a point where it will be practical for wide-scale culturing for human type tubercle bacillus.

Summary

At Muirdale Sanatorium, a hospital of about 600 beds, gastric aspiration cultures have proved to be one of the most trustworthy laboratory tests in diagnosing the early symptom-free cases of tuberculosis and in determining when the treated can safely be discharged from the hospital. Our own success suggests that culture of fasting stomach contents should be given a place in the bacteriological laboratories of general hospitals. These institutions would thereby be contributing toward a more concerted effort to check the spread of tuberculosis.

Note

Grateful acknowledgment is made to my co-workers, Miss Mary K. Warren and Miss Zita M. Pieper, without whose valuable assistance this work could not have been accomplished.

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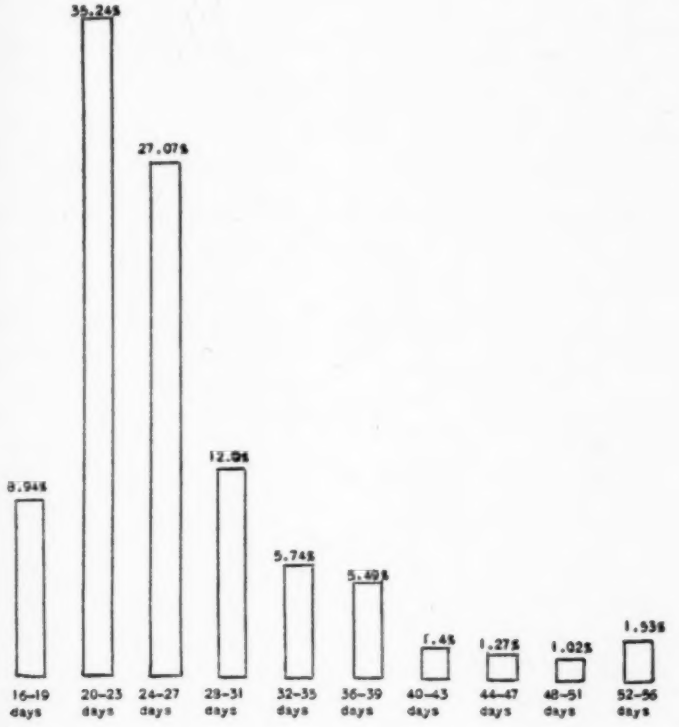
DISTRIBUTION OF POSITIVE GASTRIC ASPIRATION CULTURES



About 89% of positives are reported by end of 5 weeks.

25 days is the average period required for a positive culture.

(Based on a study of 783 positive cultures on Muirdale Sanatorium patients from Jan., 1942 to Jan., 1944, inclusive.)

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ADMISSION HEMATOLOGY*

An experiment by PAULINE KURACHI, B.A., M.T. (ASCP)
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The study of blood goes back into obscure ancient history, but in 1673, following his invention of the microscope, Leeuwenhoek (1) began the study of red blood cells. In 1852, Vierordt (1) and in 1854, Walker (1) ingeniously made the first blood counts. In 1890, Hedin (1), Blix (1) and in 1891, Daland (2) introduced the hematocrit which has become popular in the past few years. In 1895, Sahli (1) used the acid hematin method for the determination of hemoglobin with the use of 0.1 N. hydrochloric acid. In 1904, Arneth (1) introduced the classification of neutrophils. In 1918, Fahraeus (3) found that the suspension stability of blood was altered in many conditions. In 1929, Wintrobe (4) described methods for determining the size and hemoglobin content of the erythrocyte. There have been other important developments in hematology but a complete outline of the history of hematology is outside the scope of this paper.

Admission hematology has long been desired for patients entering this mental institution. The patients are not acutely ill and they are often resident for appreciable periods of time. Since it was not known whether admission hematology could be handled under existing conditions and since only comprehensive studies could be of real value in this branch of medicine in which many factors remain unknown, a very complete routine hematology was set up as an experiment, and thus far has proved valuable in stimulating us to a thorough study of hematology and in giving us more concrete technical checks.

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*Honorable mention, June, 1945.

Scope

The admission routine, begun July 5, 1944, consists of the determination of the following: the total erythrocyte count, the hemoglobin determination, the volume of packed erythrocyte, the mean corpuscular volume, the mean corpuscular hemoglobin, the mean corpuscular hemoglobin concentration, the erythrocyte abnormalities such as anisocytosis, poikilocytosis, and chromia (observed in smears), the parasites, the sedimentation rate, the corrected sedimentation rate, the buffy coat layer either as one layer or two layers, the total leukocyte count, the differential cell count including the Schilling classification of neutrophils, the platelets as normal, increased or decreased (by the stained smears) and the reticulocyte count.

Material

Venous blood is used entirely and 5 cc samples are taken for the complete examination. Certain precautions are observed in obtaining the venous blood; the tourniquet is not applied until all is in readiness for doing the venipuncture, after obtaining the blood the needle is removed before emptying the syringe, and the blood and anticoagulant are thoroughly mixed by rotation of the stoppered bottle and formation of foam is avoided. No study is made on blood which shows hemolysis or clots due to improper technic in securing or handling the venous blood, but another sample is taken.

The bottle, in which the blood is taken, is a wide mouth specimen bottle, flint glass, squat form, cork stoppered, 30 cc capacity. This size and type of bottles have the advantages that not only no blood comes in contact with the corks and the corks can be replaced readily to prevent evaporation; but also they are easily rotated, and readily allow the pipettes to be passed to the bottom.

The anticoagulant used is a dry mixture of ammonium and potassium oxalate. For the 5 cc of blood 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate are used. This mixture is obtained by dissolving 1.2 grams of ammonium oxalate and 0.8 gram of potassium oxalate in 100 cc of neutral distilled water (1 cc of 40% formalin added for preservative) and placing 0.5 cc of this solution in each bottle and drying. The ammonium oxalate causes swelling of the red corpuscles while the potassium oxalate leads

to shrinkage. The ratio in which they are used is that recommended by Wintrobe (1) as being equivalent to heparin, which causes no cell alteration. We have had no experience with heparin; it is more expensive and though it does not alter the size of the corpuscles, according to Wintrobe (1) it does alter the sedimentation rate and the salt mixture does not.

Procedure

The number of patients to be examined so completely required a preliminary systematization and organization by the pathologists and medical technologists since for this undertaking there had been no proportionate increase in the laboratory personnel. Under these circumstances it would have been impossible to consider handling hematological studies on all patients as admitted had they to be done daily and singly; but with all patients admitted during the week examined on one day, the work could be organized, systematized and attempted. The pathologist already had a routine established doing all the lumbar punctures and venipunctures for serology on one day a week and at that time an additional 5 cc of blood is easily taken. In the laboratory the day was already reserved to some extent for admission spinal fluid examinations and preparation of bloods for the following day's serology, and by rearrangements throughout the week a clearance was made for admission hematologies.

The day before the hematology day the lists of the names of the newly admitted patients are sent to the pathologist, and from these lists the bottles and complete hematology report sheets are properly labeled.

On the hematology day while the venipunctures are being done on the ward, the work-table (Fig. 1) in the laboratory is prepared as follows: (a) Wintrobe hematocrit (blood volume index) tubes are numbered to correspond to the numbers of the oxalated blood samples and placed in an upright rack. These tubes are set up for the sedimentation rates which are recorded at the end of the hour, and the filled hematocrit tubes are then used for the packed cell volumes. A rubber bulb is put on each hematocrit pipette making it ready for immediate use. (b) Colorimeter tubes are numbered to correspond to the number of the oxalated blood samples, set up in a rack, and 10 cc (measured in a standardized volumetric

pipette) of 0.1N hydrochloric acid is pipetted into each tube, which is then stoppered to prevent evaporation at this altitude and climate. These tubes previously calibrated are for the photoelectric acid-hematin method of hemoglobin determination. (c) Clean slides and medicine droppers are arranged conveniently for making differential smears. (d) All the diluting pipettes for both red and white corpuscles previously cleaned in dichromate solution are placed ready for use along with Hayem's fluid and 1% acetic acid solution. The counting chambers and covers are thoroughly cleaned and set up. Counting chambers used are either certified or have been checked with a certified one.

The blood specimens are sent in lots to the laboratory. In each half of the day regardless of the number of patients to be examined, not more than 16 samples of blood are collected. In our experience this is the present maximum capacity of our laboratory personnel. Our centrifuge accommodates 16 hematocrit tubes, which is another reason for not handling more than 16 samples each half day. All of these bottles of blood samples are kept tightly stoppered with a cork stopper and opened only during pipetting of the blood. Before uncorking, the contents are always thoroughly mixed by rotating the bottles on the table surface. The amount of shrinkage due to evaporation during pipetting only is negligible.

Two technologists can handle all the preparations and the mechanical and technical procedures of the examinations. The third technologist makes the differential slides in duplicate within the first hour, she is otherwise free to handle the unrelated work coming in.

The sedimentation rate test is set up first, noting time of the hour. An interval timer is used and if the test is done by one technologist, after some experience one hematocrit per minute can be set up. The tubes are read in the same order at the end of the hour, and the one minute interval is found sufficient time for reading and recording the sedimented column.

All hemoglobin dilutions, red cell dilutions and white cell dilutions are made consecutively. There is no change in these structures in the sample during the first 24 hours, so in spite of the number of examinations each may be done carefully and meticulously, as is necessary for accuracy. A pipette shaker would facilitate the counts, but one is not available as yet.

After all the hemoglobin dilutions have been made the racks of tubes are removed to the photoelectric colorimeter and the hemoglobins determined and recorded after the last hemoglobin dilution has stood at least three minutes.

At the end of the hour sedimentation rates are recorded and the filled hematocrit tube is then placed in the centrifuge and spun at 3,000 r.p.m. for 30 minutes. Then the packed erythrocyte volume is read from the hematocrit tube scale and the leukocyte and platelet volume are read as accurately as possible. The volume of leukocytes and platelets within normal range is so small compared to the volume of erythrocytes, that only an estimate reading can be made. The leukocytes and platelets are in a reddish-gray layer above the erythrocyte layer and are recorded as buffy coat. If the buffy coat has two layers instead of one, a rough estimate of each layer is made and recorded. The cell volumes and sedimentation rates must be done during the first three hours (5).

The blood smears for differential study and counts are always made in duplicate and stained during the first hour. Differential smears made after an hour's time are not satisfactory for study or differential counts (5).

Wintrobe recommends the use of coverslip preparations for differential counts. This method, in which a small drop of blood is placed on a clean coverslip, covered with another clean coverslip, the blood allowed to spread between them, and then the two coverslips drawn quickly apart, has been used at the Colorado State Hospital for a number of years. However, the cost of coverslips is high, and their quality has become poorer, so that it has been necessary to discard many more coverslips than formerly because they contained bubbles or were uneven or scratched. Also coverslips could not be used again for making blood smears after they had been stained because any extraneous matter on the surface of the coverslip interfered with the even spreading of the film of blood. Finally, stained coverslips must be mounted on glass slides. This mounting takes time, and the slides used must be washed. For these reasons it was decided to change to the glass slide method for making blood smears for differential counts in this experiment.

However, as soon as the change was made all the patients' reports showed a relative lymphocytosis, and it was found that the

white blood cells were not distributed evenly over the surface of the slide. The heavier cells, notably the polymorphonuclear neutrophils, were clustered at the sides and ends of the smear, and the lymphocytes which are lighter in weight, were concentrated in the center of the smear.

It was found that the faster the smear is made, the more even the distribution of the white cells throughout the smear, and the closer is the general agreement of results with those commonly found here by the coverslip method. A small drop of blood is pushed quickly across the surface of the slide without waiting for the blood to spread from side to side of the pusher slide. Smears made in this way appear to be much thicker than the ones which are made more slowly, but examination under the microscope shows that the white cells are distributed evenly throughout the smear, and are spread thinly enough to study cytologically and to count with ease and that there are areas in which the erythrocytes can be studied individually.

Calculations

From the volume of packed erythrocytes, erythrocyte count and hemoglobin, the size and hemoglobin content of the erythrocytes are determined. The mean corpuscular volume (MCV) is the calculated size of the patient's red corpuscles expressed in cubic microns. The mean corpuscular hemoglobin (MCH) is the calculated weight of the hemoglobin in the average corpuscle expressed in micromicrograms. The mean corpuscular hemoglobin concentration (MCHC) is the calculated concentration of the hemoglobin in the average cell expressed in percent.

The mean corpuscular volume is calculated according to the following example:

$$\frac{\text{Volume of packed erythrocyte per 1000 cc}}{\text{Erythrocyte count, millions per cu. mm.}} = \frac{450}{5.0} = 90 \text{ cu. microns}$$

Volume of packed erythrocyte per 100 cc = 45 cc
Erythrocyte count = 5.000,000
The normal range = 82 to 91 (1)

The mean corpuscular hemoglobin is calculated as follows:

$$\frac{\text{Grams of hemoglobin per 1000 cc}}{\text{Erythrocyte count in millions per cu. mm.}} = \frac{157}{5.} = 31$$

Grams of hemoglobin per 100 cc = 15.7
The normal range = 27 to 31 (1)

The mean corpuscular hemoglobin concentration is calculated as follows:

$$\frac{\text{Grams of hemoglobin per 100 cc}}{\text{Volume of packed erythrocyte, cc per 100 cc}} \times 100 = \frac{15.7 \times 100}{45} = 35$$

The normal range = 32 to 36 (1)

The absolute values obtained for the blood picture from the just mentioned formulas have definite normal ranges and abnormal limits. When these are exceeded a technical error or faulty standards or a very remarkable case is known to exist; to check for technical error, the hemoglobin determination and erythrocyte counts are retaken on the original blood sample. If no error in those is revealed, complete re-examinations are done the following week on hematology day. Repeats on cell volumes and sedimentation rates are not usually done on the original sample because it is difficult to do the checks within the allowed three hours (5).

The thickness of the buffy coat layer depends upon the number of leukocytes, the kind of leukocytes and number of platelets (1). The buffy coat is normal between 0.5 and 1 mm. thick since about 1,000 leukocytes are 0.1 mm. thick. Although it is recognized that this correlation is not absolute and is less accurate above 12,000, we repeat all leukocyte counts which do not correspond to that which might be expected from the buffy coat reading.

Rechecks on erythrocyte count, leukocyte count and hemoglobin are done throughout the day. These rechecks may be done within 24 hours (5). Tests giving quite unusual findings are always repeated. This is no great inconvenience as the original blood sample is at hand. Among the unusual (rechecked) findings there were two cases of polycythemia, in 580 cases: only one was further studied since the other case died after one hematological study in which he had an erythrocyte count of 8,170,000 with a packed erythrocyte volume of 64 and hemoglobin of 21.5 grams.

The case still under study has had the following rechecked bizarre findings:

Date	*Cell Volume*	*Hemoglobin*	*RBC*
Dec. 15, 1944	61	18.6	5.33
Jan. 11, 1945	63	22.	6.38
Feb. 15, 1945	69	20.5	6.35
March 1, 1945	62	22.	6.62

Technical Checking Value With Packed Cell Volume and Absolute Measurements

Though the buffy coat reading is not an alternative to the leukocyte and platelet count, in our experience it definitely affords a rough estimate of leukocytes and possibly lymphocytes and platelets present. If a buffy coat layer measuring 2 mm. had a leukocyte count of 10,000 or if a buffy coat layer measuring 0.5 mm. had a leukocyte count of 13,000, these would definitely require a recheck on the leukocyte count. In the 392 cases only eight rechecks of leukocyte count were indicated by lack of correlation with the buffy coat. In four of these eight cases no inaccuracy was found on recheck of the leukocyte count; in the other four cases the rechecks showed the original counts erroneous and the correct count in rough agreement with the buffy coat reading.

Up to the present no admission case has had a spectacular leukocytosis and high buffy coat. The two most outstanding cases of leukocytosis with an above normal buffy coat are: Case I: buffy coat one layer 2.5+mm. and leukocyte count 30,000; Case II: buffy coat one layer 2.0 mm. and leukocyte count 21,500.

According to hematologists, the presence of two layers in the buffy coat layer may designate numerous platelets. Narrower reddish gray layer may indicate presence of lymphocytes and platelets. Although we could not always demonstrate such relationships, we occasionally had striking examples. The following are two cases showing two layers in the buffy coat layer: Case I: Buffy coat, total 0.6 mm. with 0.3 mm. in the first layer and 0.3 mm. in the second layer, leukocyte count 8,400 with 46% lymphocytes, and 48% segmented neutrophils. Case II: Buffy coat, total 0.5 mm. with 0.25 mm. in the first layer and 0.25 mm. in the second layer, leukocyte count 7,400 with 41% lymphocytes, and 49% segmented neutrophils. The platelets appeared normal in the stained smear in both cases. These illustrate the predominance of lymphocytes by the presence of two layers.

The reading of the buffy coat layer and its correlation with the leukocyte count serve as quite elastic checks. The range of possibility is greater than that of the erythrocyte, and the packed erythrocyte volume. An error in erythrocyte count is indicated and the erythrocyte count is repeated when according to the absolute values,

Fig. 1. Work Table.

NAME Doe, John Wd. 6 Dr. A.B.F. HEMATOLOGY Physiological
Adm Date 4-1-45 At age 70
Mental Diag Senile Psychosis Clin. Diag Chronic Myocarditis

Date 4-17-46 No. 22

RBC	Hgb gm 17.2	SED RATE 12	Corrected 13	Platelets ✓
116	VOL. RBC 48	Buffy (1) 0.25	JUV	% WBC
112	MCV 86	coat (2) 0.25	STAFE 111	2 % 46
108	MCH 31	SEGS [illegible]		56 % 39
110	MCHC 36	[illegible]	BASO	% 41
113	Anis 0	EOSIN 1		1 % 40
	Poik 0	LYMPH [illegible]		37 % 116
5.59	Chromia ✓	MONOS [illegible]		3 % 8300

Clin Diag Wd Dr HEMATOLOGY

Date No.

RBC	Hgb gm	SED RATE	Corrected	Platelets
	VOL. RBC	Buffy (1)	JUV	WBC
	MCV	coat (2)	STAFF	%
	MCH	SEGS		%
	MCHC		BASO	%
	Anis	EOSIN		%
	Poik	LYMPH		%
	Chromia	MONOS		%

Fig. 2. Patient's Record Sheet.

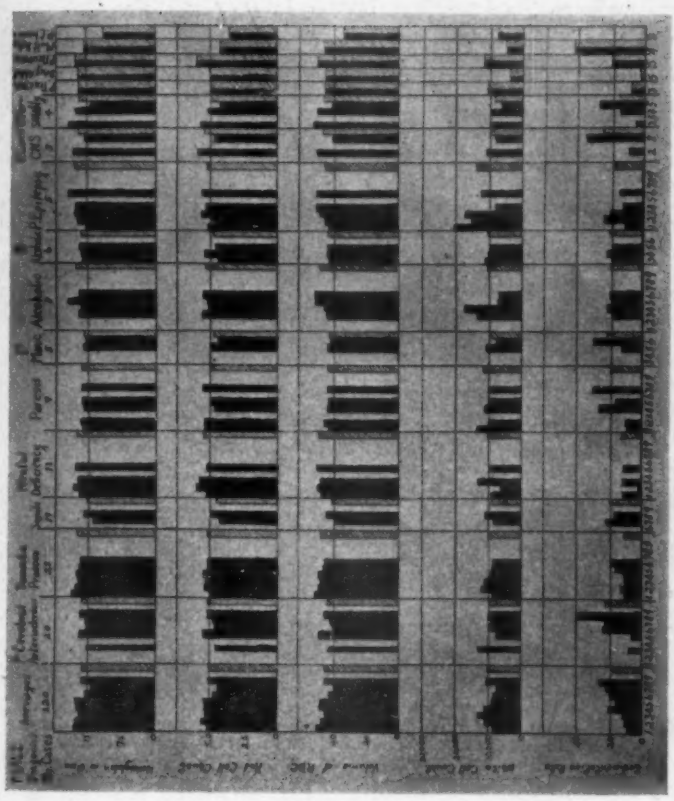

Fig. 3. Age in Ten Year Periods—1=10-20 Years, 2=20-30 Years, Etc.
Shaded Parts—Group Averages.

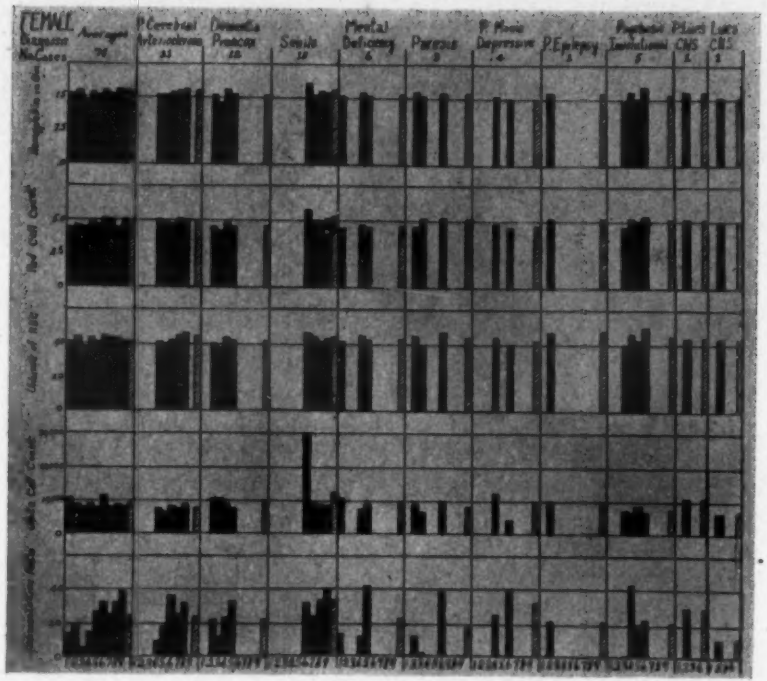

Fig 4. Age in Ten Year Periods—1=10-20 Years, 2=20-30 Years, Etc.
Shaded Parts—Group Averages.

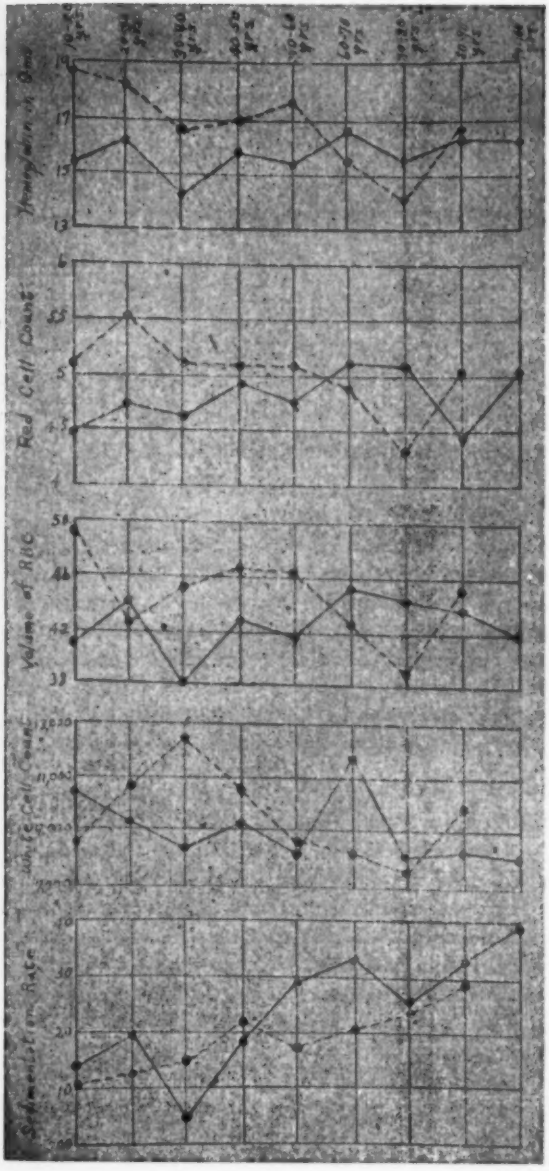
Hemoglobin in Gms.
Red Cell Count
Volume of RBC
White Cell Count
Sedimentation Rate

Fig. 5.

the erythrocyte count appears to be too high or too low or when the patient's hematological picture changes radically and bizzarely, as in the following example. This particular incident occurred in a series of hematological studies on a patient whose findings were consistently those of macrocytic anemia. In this cited incident the volume of packed cells was 43, the first count gave 5,500,000, the second count 5,300,000, the third count 4,490,000 on the same sample of blood. In the course of treatment the patient had shown definite improvement, but the picture given by the 5,500,000 erythrocyte count showed a microcytic picture (MCV 78, MCH 28, MCHC 37) instead of an improved macrocytic picture as does the 4,490,000 count (MCV 96, MCH 35, MCHC 37).

While the above contradictions in the hematological picture are usually brought to inexperienced technologists' attention by the pathologist, it is easy for any technologist to see that a possibility of error in hemoglobin determination has to be ruled out whenever the MCHC is above a certain level. This is the first and easiest check to make. It is understood that there is a maximal concentration of hemoglobin in the erythrocyte and this maximal concentration is maintained in normal cases and some anemias; a reduction below the normal maximum concentration is the only direction in which the blood can change (1). At this institution this maximal concentration was found to be 39% according to our hemoglobin method. Since 37% is the highest value given for the MCHC it is obvious that our hemoglobin determination is high. This determination is made from a chart supplied by the makers of the photoelectric instrument. We have not yet obtained the chemicals or equipment necessary for making our own calibration curve.

It is apparent that when here a MCHC of 40 or over exists, the hemoglobin and/or the packed erythrocyte volume must be checked. For example, in a case where our MCHC was 40, two checks on an 18.2 hemoglobin showed the correct value to be 17.0 grams changing the MCHC to 39. Forty-three rechecks on hemoglobin determination of 392 cases showed twenty original hemoglobin determinations were erroneously high. If the recheck on the hemoglobin does not alter the MCHC from 40 or above, then the cell volume must be checked. In such a case another sample of blood should be secured from the patient, because the cell volumes in the

original samples change. From 392 cases two cases out of eight, checked for packed erythrocyte volume, changed the calculation of MCHC from 40 to 39 and 37. In the other six cases the MCHC remained unchanged, though the hemoglobin and packed erythrocyte volume had changed.

By this type of routine hematology study another type of error was picked up which would not have been detected if the erythrocyte count and hemoglobin determination alone were done; the error was that of interchanging the hemoglobin tests. Patient A had Rbc 4,050,000, hemoglobin 13.8 grams, cell volume 29, MCHC 47+; Patient B had Rbc 4,200,000, hemoglobin 10.8 grams, cell volume 39, MCHC 28. The hemoglobin in either case would be possible for one or the other, but in calculating the absolute values, Patient A has a hemoglobin which gives MCHC as 47+, an impossible result; while Patient B presents a picture of extreme hypochromia, a patient who has been under treatment for macrocytic anemia. In checking the hemoglobin it showed clearly that the hemoglobin had been interchanged, and the MCHC for Patient A became 37 and the MCHC for Patient B became 35.

Errors

All errors, except the constant and limited one of a high MCHC limit, have been technical. Tubes, pipettes, everything, came under suspicion but when tested all instruments were found accurate.

While the errors have constantly been technical, the technical errors have not been constant. There are days in which the percentage of technical error is greater than on other days. It has been found that those days having a larger number of blood samples require a higher percentage of rechecks. This is believed to be attributable to the strain placed upon the technologist going at top speed. This, however, may not be true of each individual.

The error most frequent is the inaccurate filling of the hemoglobin pipette. In some instances as much as 1.2 gram error was found. Such other errors as have been revealed have not been so frequent and have not been fully analyzed.

In this group of 480 cases studied and checked and rechecked according to the foregoing routine, 50, regarded as normal, form the basis of the following figures given in comparison to recognized

standards; these conclusively show that our hemoglobin determinations are too high.

	Colorado State Hospital	*Wintrobe*	*Kolmer*
MCV	82—93	82—91	82—92
MCH	31—36	27—31	29—31
MCHC	35—39	32—36	33—37

We look forward to correcting the hemoglobin determination and obtaining results which can be judged by universal standards instead of our above local standards for normal.

Method of Recording Blood Findings

With the institution of routine admission blood examinations and the addition of new hematological data it was necessary to work out an individual report sheet for the blood findings which could be filed separately so as to be available to the laboratory force for study and reference, making possible the foregoing and subsequent evaluations.

The first problem was to devise a system which would involve no additional record work. This was accomplished by working out a record form (Fig. 2) which was compact enough to be used at the microscope for the direct counting of the red and white blood cells and for the differential count, and which provided spaces for recording the hemoglobin, sedimentation rate and packed cell volume, as well as space for the corrected sedimentation rate and the Wintrobe indices, but which was clear enough that it could be filed as the permanent duplicate record. At the top of the hematological form were spaces for the name of the patient, the date of his admission to the hospital, his age, mental diagnosis, the clinical diagnosis at the time of the count, and the hematological diagnosis based on the laboratory findings.

The advantages of this new system are apparent. Not only is time saved by the elimination of one step in the recording of blood counts, but the chance of errors in copying reports is less. By having these data on a separate but permanent record, it is possible to classify them hematologically; a colored tab attached to the reports is used to identify reports showing various types of anemia, a tab of another color identifies those showing a high white count. These colored tabs could be used in the same way to identify reports according to any cross classification without disturbing the

alphabetical order of the files. Although filed alphabetically for general purposes, the size of the forms lends them to rearrangement according to any project at hand, such as was done subsequently in the study of variation in age groups and mental diagnosis.

Absolute Measurements and Recorded Appearance of Erythrocytes

The use of these complete report sheets as work sheets for recording the differential counts as they are made has led to some interesting comparisons between the blood picture according to the Wintrobe indices, which are calculated from the red cell count, the hemoglobin in grams, and the packed cell volume, and the actual appearance of the red cells under microscope on a stained blood smear.

In the three-month period from January 18, 1945, to April 16, 1945, thirty cases of anemia were found in the studies. The hematological diagnosis of anemia is made on the basis of the red cell count, the hemoglobin content of the blood and the Wintrobe indices. Of these thirty cases, sixteen were "normocytic" indicating that the cells were of normal size, and contained a normal amount of hemoglobin, but the total number of cells was smaller than normal. Five cases were "microcytic" or "hypochromic," that is the cells were small in size and/or deficient in hemoglobin, but not necessarily small in number. Nine cases were "macrocytic" or "hyperchromic." In these, the cells were large in size and had a higher than normal amount of hemoglobin in each cell (a high MCH) but the concentration of hemoglobin (MCHC) was normal; the red cell count was usually very low. The red cell counts for the series ranged from 2.25 to 4.97 million per square millimeter, and the hemoglobin from 7.85 to 16.4 grams per 100 cc blood. In all of these cases the size, shape and color of the red cells on stained smears was observed and reported when the differential count was made. In only fifteen of the thirty cases was a difference from the normal appearance of the red cells noted.

In all five of the cases of microcytic anemia the cells appeared to be hypochromic, in four of the five cases there was variation in size and shape of the red cells, and in two of the five cases the red cells were noticeably smaller than normal.

In two of the nine cases of macrocytic anemia some of the cells appeared hypochromic, while, according to the Wintrobe indices, the mean cell actually contained a normal or higher than normal amount of hemoglobin. In only one of the nine cases was the larger size of the red cells sufficiently apparent that it was noted.

Of the sixteen cases of normocytic anemia, five had red cells which appeared hypochromic under the microscope, and in one polychromatophilia was noted. In three of the sixteen cases a variation in the size and shape of the red cells was observed.

From these figures it is apparent that there is not always strict agreement between the calculated values for the size, amount of hemoglobin, and hemoglobin concentration in the individual erythrocytes and the appearance of the red blood cells on stained smears. In fact, in routine hematological study a noticeable change in the appearance of the red cells was observed in only half of the cases of anemia.

Classification of Data

It was the desire of the personnel of the laboratory to know whether or not any data of interest in relation to mental disease could be found by studying the results of the routine hematological studies of the newly admitted patients. For this purpose, it was necessary to have the mental diagnosis on the hematological record. The hematology is done within the first week after the patient's admission, but not until a week or two after the laboratory work is done is the mental diagnosis officially made, and often not until this time are the ages of those patients not able to give their age obtained from the relatives. The recording of diagnosis and age on the hematological form is therefore done periodically rather than routinely. The forms, completed with the mental diagnosis, were sorted into their respective mental disease groups. Since it has long been noted that the sexes show a difference in the blood picture and that there is also a difference according to age, the groups were subdivided into male and female and each of these subgroups subdivided into 10-year period age groups.

This study concerns the hemoglobin, red blood cell count, cell volume, sedimentation rate and white blood cell count in the above groups and subgroups. The low average and high of these hemato-

logical values were determined for the group as a whole and for each disease group, male and female and 10-year age period; these averages were tabulated.

From these tabulations were made two block graphs, one for male (Fig. 3) and one for female (Fig. 4) in which graphs age-groups and mental disease groups were charted for hemoglobin, rbc, cell volume, sedimentation rate and wbc. A linear graph (Fig. 5) was made comparing these values in male and female in the 10-year period age groups.

Analysis of Classified Data

Hematological studies have been made by various workers all over the United States, mostly at sea level. Mugrage and Andresen (6) have made a study in Denver, Colorado, of 40 normal men and 40 normal women. Since Colorado State Hospital in Pueblo is nearly the same altitude as Denver, their values are used as normal in our classification. Below is a comparative chart showing their normals' values and the Colorado State Hospital patients' values, based on 193 admissions; 73 females and 120 males, unselected, and diagnosed between September, 1944, and March, 1945.

	Mugrage-Andresen			*Colorado State Hospital*		
	Hgb.	Rbc.	Cell Vol.	Hgb.	Rbc.	Cell Vol.
Male	16.87	5.42	48.33	16.11	5.04	45.26
Female	14.33	4.63	43.22	15.82	4.81	42

It is easily seen that these agree relatively closely.

By mental diagnosis the patients in this study fall into 17 different groups as represented in graphs 1 and 2. In these block graphs the one thing that stands out in the mental diagnosis groups is that there is no great difference in the general hematological pictures, however, within these groups are seen interesting subgroups which have the following values conspicuously above the average:

(a) Hemoglobin and rbc for both sexes between ages 40-50, and psychosis, males, 30 to 50 years of age; and in epileptics, males, 60-70 years.

(b) Red blood cell count in mental deficiency, male and female, 20-30 years; and in psychosis with syphilis of the central nervous system, male and female, 20-30 years.

(c) Cell volume in involutional psychosis, female, age 60-70.

(d) Sedimentation rate in psychosis-cerebral arteriosclerosis, male, age 80-90; mental deficiency, female, age 50-60; and psychosis involutional, female, 30-40.

(e) Leucocytes in alcoholics, both psychosis and without psychosis, male, 30 to 50; and in epileptics, male, age 10-20.

In the linear graph, the one thing that stands out is that all values for females are higher than for males in the 60-70 and the 70-80 age groups. This is a reversal of the usual relationship and the relationship which we found up to that age. A preliminary uncontrolled study by one of us (W.R.H.)* revealed the same reversal of male and female relationships in the older age groups. Other points of interest in the linear graphs are the following lowering of the curves for:

(a) Hemoglobin and rbs for both sexes between ages 40-50, and again at ages 70-80;

(b) All blood values (except sedimentation rate) for both sexes between ages 80-90;

and a decided rise in the curve for the average white count of men 30-40 years of age.

No statistical validation or formulation has been made and therefore no conclusions are drawn from these observations.

Summary and Conclusion

The organization, technical procedures, methods of recheck, recording, classification of data and analysis of results of a routine admission hematological system have been presented.

This system, set up experimentally, covering erythrocyte count, leukocyte count, hemoglobin determination, sedimentation rate, packed cell volume, Wintrobe indices, and form for recording these data, has been feasible in practice and of benefit to the laboratory personnel in providing checks on the technical procedures employed and in stimulating a more complete study of hematology.

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*"Routine Hematology In the Clinical Laboratory"—Paper read at 1944 Colorado State Medical Technologists Convention.

The authors express their appreciation to the pathologists Dr. Carl W. Maynard and Dr. Mae Gallavan for their leadership, and to Miss Du Void Burris for her assistance with the illustrations.

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CLINICAL EVALUATION OF LIVER FUNCTION TESTS

By K. Y. YARDUMIAN, M.D.

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Every clinical pathological laboratory in this country has its own favorite tests for liver function. We have been trying some of those tests in our own laboratory for the last several years to see for ourselves how much help we can receive in diagnosis and prognosis of liver diseases.

Within the last two decades a dozen or more tests have been recommended for functional study of the liver. Some already have been discarded and others are in the process of evaluation; and few are still in use with fairly satisfactory results but not too conclusive. The reason why most of these tests have been rather disappointing is that we are dealing with an organ with numerous known functions and perhaps many more unknown functions. Furthermore, enormous reserve capacity and remarkable regenerative powers of the liver is very well known. It has been estimated that about 75% of the liver must be destroyed anatomically or functionally before gross impairment of its functions occur.

If we accept this dictum, then one wonders whether there are any tests that will enable us to detect liver damage when less than 75% of the organ is incapacitated.

In order to evaluate the liver function tests, let us just review very briefly the most important functions of the liver: (1) carbohydrate, protein, and lipoid metabolism; (2) formation and excretion of bilirubin, biliary salts, porphyrins, phosphatase; (3) detoxification and conjugation of amino acids and other toxic substances; (4) important storehouse for iron, anti-anemic principle, fat soluble Vitamins A, D, and K; (5) reservoir of venous blood; (6) elimination of certain dyes; (7) important center of heat production.

I will mention some of the liver function tests recommended for detection of liver damage: (1) urine: bilirubin and urobilinogen

and study of the sediment of leucine, tyrosine and cystine crystals; (2) carbohydrate metabolism: glucose, galactose, levulose, and lactic acid tolerance tests; (3) protein metabolism test: albumin-globulin ratio, cephalin flocculation test, prothrombin, Takata-ara and colloidal gold tests; (4) conjugation or tests for synthesis: hippuric acid test and cinchophen oxidation tests; (5) excretion and elimination tests: bromsulfalein, rose bengal, Congo red, bilirubin and urobilin elimination tests; (6) retention of biliary pigments: icterus index and Van den Bergh tests; (7) fat metabolism tests: cholesterol and cholesterol esters.

Contraindications and difficulty of interpretation of some of the tests: The carbohydrate tolerance tests mentioned above have little value in patients with suspected liver damage, with diabetes and other endocrine dysfunctions; Hippuric acid test is contraindicated in renal diseases and congestive heart failure. Most of the elimination tests are valueless in cases of obstructive jaundice. Cephalin flocculation test is too sensitive and the results are transitory. In most systemic infections, cephalin flocculation test may give a positive result without serious damage to the liver. However, this positive reaction may be due, to some extent, to toxic hepatitis.

The detailed discussion will be confined to cephalin flocculation, hippuric acid, and bromsulphalein elimination tests. I wish to emphasize the common errors in performing the test.

Hippuric Acid Elimination Test

This test originally was introduced for kidney function test by Lewis, Kingsbury and Bell. More recently (1925) Bryan, in attempting to use this test as one of the kidney function, showed that hepatic damage in dogs produced a decrease in the amount of available glycine in liver. In 1933, Quick, on feeding sodium benzoate to nephrectomized rabbits, hippuric acid was found in blood, liver and muscles.

In regard to the mechanism of this synthesis, it is known that hippuric acid is one of the end products of protein metabolism and is a combination product of glycine and benzoic acid. This synthesis takes place in normal liver and consequently poor synthesis indicates damaged liver.

The original technique of the test by Quick is as follows: 5.9 grams of sodium benzoate (equivalent to 5 grams of benzoic acid) dissolved in 30 cc. of water are administered one hour after breakfast consisting of coffee and toast. The patient is then given one-half glass of water. Immediately after taking the drug the patient voids, and then collects complete hourly specimens for four hours. These are preserved with Toluene, and hippuric acid determined in each specimen. Later it was found too cumbersome and time consuming to collect hourly specimens and estimate the amount of hippuric acid in each hourly specimen. After all the total four-hour elimination was taken into consideration in estimating the liver damage. Subsequently the oral administration of sodium benzoate was replaced by intravenous technique to avoid the gastrointestinal irritation manifested by nausea, vomiting, diarrhea, and anorexia, and also to shorten the period of collection of urine from four hours to one hour.

The intravenous technique consists of administration of 20 cc. solution containing 1.77 grams of sodium benzoate equivalent to 1.5 grams of sodium benzoate intravenously. The morning of the test let the patient have a light breakfast and approximately one hour after the bladder is emptied and discarded, exactly one hour after the intravenous injection of the sodium benzoate solution, another specimen is collected and sent to the laboratory. During the test no water is allowed.

At the laboratory the urine specimen is measured and transferred to a beaker and acidified with concentrated hydrochloric acid until acid to congo red; one cc. of the acid is sufficient. The solution is vigorously stirred with glass rod by scratching the bottom of the beaker, and then allowed to stand for an hour at room temperature. The precipitate is filtered off on a small Buchner filter plate, washed with a small quantity of cold water, and allowed to air dry. The hippuric acid thus obtained is weighed to the second decimal place, which is usually sufficient. To obtain the total hippuric acid, one must add to the amount thus obtained, the calculated quantity remaining in solution; 100 cc. of urine will dissolve 0.33 grams of hippuric acid. In case a specimen exceeds 125 cc. it should be slightly acidified with acid and concentrated on water bath to about 50 cc. before precipitating the hippuric acid. The results are

best expressed in terms of benzoic acid. To convert hippuric acid to benzoic, one multiplies by 0.68.

The normal elimination within four hours ir terms of benzoic acid should be over 3 grams. While in intravenous technique one hour elimination should be over 0.75 grams. This test is counter-indicated in advanced kidney diseases and cardiac decompensation, and has no practical value since there is very little or hardly any elimination. Patients with extreme jaundice generally eliminate large amounts of biliary pigments. In running this test the bile pigments are precipitated with HCl and will falsify the quantity of ppt. In these cases powdered charcoal is recommended to precipitate off the biliary pigments before adding HCl.

The toxic symptoms of benzoic acid are two kinds: (1) those due to local irritation gastrointestinal mucosa, with nausea, vomiting, diarrhea and anorexia; (2) central nervous system symptoms with headache, tinnitus, vertigo, giddiness and reflex vomiting.

Add 5 grams of ammonium sulphate for each 10 cc. of filtered urine. Dissolve by holding in warm water if necessary. Make distinctly acid (deep blue to Congo red paper) with concentrated hydrochloric acid, and stir with a glass rod, scratching the sides of the flask to start crystallization. Let stand one-half hour. If no precipitate is formed in this time, leave overnight at room temperature.

Filter with suction through a dried and weighed fritted glass filter, washing the flask and the precipitate two or three times with 5 cc. portions of ice water. Leave overnight at 37° C. to dry. Weigh, subtract the weight of the filter and correct for the amount of hippuric acid remaining in the solution by adding 0.1 gram for each 100 cc. of solution, including the washings. If preferred, a retentive filter paper (E. & D. 613) may be used instead of the fritted glass filter, using a similar paper as a tare weight.

Cephalin-Cholesterol Flocculation Test

This test was first introduced by Hanger, 1939. The principle of the test is that certain globulins in the serum in acute liver diseases are flocculated by addition of an antigen, named cephalin cholesterol.

Technique of the Test—Reagents

Cephalin, partially oxidized 100 mg.
Cholesterol 300 mg.
(These are supplied in one tube ready for use.)
Ether U.S.P. (anesthesia).
These reagents can be purchased from Difco Laboratories, Detroit, Michigan

Prepare a stock ether solution by placing 8 cc. of ether in the vial containing the Cephalin and Cholesterol. Stopper and shake well. This stock ether solution is stable if kept in an ice box.

Prepare an emulsion by adding (with stirring) 1 cc. of the stock ether solution to 35 cc. of freshly distilled water which has been warmed to 65 or 70° C. Heat the mixture slowly to boiling and allow to simmer until the final volume reaches 30 cc. Cool to room temperature. This emulsion may be used only on the day prepared.

Place 1 cc. of this emulsion in a centrifuge tube containing 0.2 cc. of the patient's serum diluted with 4 cc. of physiologic salt solution (0.85 per cent sodium chloride). Shake the mixture thoroughly. Stopper with cotton and allow to stand undisturbed at room temperature. Readings are made after twenty-four hours and the reactions are graded in terms of 0, plus-minus, 1 plus, 2 plus, 3 plus, and 4 plus. A 4 plus reaction indicates complete flocculation leaving the supernatant liquid water clear. A plus-minus reaction designates only slight flocculation. A control tube containing 4 cc. of saline solution and 1 cc. of emulsion, without serum, should be made to test the stability of the emulsion. New emulsion must be prepared freshly on the day of the test. It is essential that clean glassware be used. Serum which has been refrigerated for one day or longer may yield false positive tests.

Bromsulphalein Test

Principle — Bromsulphalein (disodium phenoltetrabromphalein sulphonate), when injected into the blood stream, is rapidly removed by the liver and excreted in the bile.

Procedure (*Rosenthal and White*)—1. Weigh the patient and calculate the amount of dye required for the test, allowing 5 milligrams per kilogram of body weight. The body weight of the patient in pounds divided by 55 will give the exact quantity in cubic centi-

meters of the 5% solution required. 2. Inject the required amount of the dye into an arm vein, taking one minute to complete the injection, with care not to infiltrate the tissues outside of the vein (a 5% solution is dispensed in ampules by Hyson, Westcott and Dunning, Baltimore). 3. Thirty minutes after injection draw 5 cc. of blood from the opposite arm. In cases of suspected early liver disease it is also advisable to obtain an additional sample exactly five minutes after injection. 4. Centrifuge and separate serum without delay. 5. Divide serum into two halves in two test tubes of the same diameter as the color tubes of the colorimeter. To one add a drop of 10% sodium hydroxide; to the other add a drop of 5% hydrochloric acid. 6. Estimate the amount of dye retained by comparing with the series of standards. The tube of clear acidified serum is placed behind the standard in the comparator box and a tube of water is placed behind the unknown.

Interpretation.—Thirty minutes after injection the serum of normal individuals is entirely free from the dye or contains but a faint trace. Five minutes after injection an average of 35% (20% to 50%) is retained. Increased dye retention may be regarded as evidence of a somewhat proportionate degree of impairment of liver function.

Summary and Conclusion

Clinical Evaluation. No one of these tests or group of them will make a differential diagnosis as to the etiology and type of anatomical lesion that cannot be successfully made by careful anamnesis and physical examination of the patient.

Clinical Value

1. Most of the liver function tests have proven of value in acute infections of the liver.
2. Liver function tests have proven of least value in chronic liver damage, especially in determining the extent of functional incapacitation of the liver. These facts have been frequently shown at autopsy, where the tests do not collaborate with the pathological findings.
3. They have special value in the pre-operative studies of patients with thyrotoxicosis, cardiac and biliary tract diseases; and thus, enabling proper pre- and post-operative care of these patients.

4. In cases of liver damage, the choice of anaesthesia may depend on the results of the liver function tests.

5. The tests have some value in detecting secondary liver damage in extra-hepatic diseases.

6. The tests may prove of value in cases of toxic manifestations of the liver in drug therapy; i. e. arsenical reaction in the treatment of syphilis.

7. Finally, they are of prognostic value in that the progression or regression of the liver damage can be ascertained.

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DEPROTEINIZATION OF CLINICAL SPECIMENS

By A. A. HORVATH, Ph.D.

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The importance of proper dilution is illustrated by the observation that by diluting human blood serum with seven volumes of 1% sodium chloride solution, it is possible to nearly double the yield of globulin by means of an ultracentrifuge.

When a method of deproteinization is employed which entails the use of a strong acid, such as trichloroacetic acid, it is essential that the precipitate should not remain in contact with the acid for more than necessary, since otherwise glycerophosphoric acid and choline will be liberated from phosphatides, giving erroneously high phosphoric ester and NPN values.

Hydrolysis during deproteinization is also considered by some investigators to be responsible for the high blood glucose figures obtained by the usual methods. When normal blood plasma is deproteinized by means of ethyl alcohol, there is practically no glucose in the filtrate. In normal individuals the glucose is presumably bound to the blood proteins, while in diabetics it is in a free state.

It is also known that tungstic acid filtrates of blood yield considerably higher reducing non-sugar values (non-fermentable reducing substances) than filtrates obtained by the zinc precipitation method. The difference usually amounts to as much as 13-24 mg. per cent.

In 1916, Folin stated that metaphosphoric acid was remarkably effective as a precipitant for blood proteins, yielding clear filtrates which did not foam on boiling. Deproteinization by metaphosphoric acid has since become a standard procedure in the determination of Vitamin C in clinical specimens, causing no oxidation of the vitamin and no discoloration of the 2,6-Dichlorophenolindophenol reagent, representing a definite advantage over trichloroacetic acid.

Recently the E. Lilly & Co. have developed convenient techniques for the rapid determination of blood sugar, albumin in urine, and amino-benzene compounds in body fluids, by using as deproteinizing agent tablets containing sodium hexametaphosphate (U. S. Pat. 2,171,961; 2,171,962; 2,208,096).

Since neither albumoses, nor peptones or amino-acids are precipitated by metaphosphoric acid, the NPN fraction of its blood filtrates could be advantageously used for studies of certain pathological conditions, where the excess NPN is subsequently precipitated from the metaphosphoric acid filtrate by means of phosphomolybdic acid.

A. A. Horvath was born in 1886, in Kazan, Russia. A graduate of the University of Kazan, Ph.D. in biochemistry. In Russian army during World War I. Subsequently on the staff of the Vladivostock Institute of Technology and of the Peking Union Medical College, China. Came to the United States in 1927, and held research positions with Rockefeller Institute for Medical Research, the U. S. Bureau of Mines, and the University of Delaware. Present position—chief of pharmacology section of Hall Laboratories.

ABSTRACTS

DRIED EGG WHITE FOR MAYER'S ALBUMEN GLYCEROL FIXATIVE: R. R. Faulkner and R. D. Lillie, Stain Tech., Vol. 20, No. 3, July, '45, p. 99.

A 5% solution of dried egg white was prepared by suspending the egg white in 0.5% sodium chloride solution and shaking at intervals for one day. Frothing should be avoided. The solution is filtered through a small Buchner funnel using vacuum. If filtration stops, the filter paper may be changed. About 95% of the original solution should be recovered. To 50 ml. of this solution, add 50 ml. glycerol and 0.5 ml. of a 1:10,000 solution of sodium ethyl mercurithiosalicylate. A clear faintly opalescent solution should be obtained.

PEPTIC ULCER DISAPPEARANCE AFTER FEEDINGS OF NORMAL HUMAN GASTRIC JUICE: L. M. Morrison, Am. Jr. Diges. Dis., Vol. 12, No. 10, Oct., '45, p. 323.

Normal gastric juice obtained by histamine stimulation was neutralized, filtered and preserved with tricresol. This was fed to patients with uncomplicated peptic ulcer. Relief of symptoms as well as negative X-ray findings were obtained. This supports the theory of a protective principle present in normal gastric juice but not in the ulcer patient.

HEMOBLOBIN VALUES FOR 2,205 RURAL SCHOOL CHILDREN IN FLORIDA: O. D. Abbott, R. O. Townsend and C. F. Ahmann, Am. Jr. Dis. Children, Vol. 69, No. 6, June, '45, p. 346.

Determinations were made with the Fisher electrohemometer. Subjects were white children from 6-18 yrs. of age. In both sexes there was a gradual increase in Hgb from the age of 6 until puberty. At 12 yrs. the values for girls exceeded that for boys. At 17 yrs. the mean value for boys was 11.45g. and for girls 11.74g. The finding of 42.3% in the group with values from 3.6 to 11.4g. was attributed to dietary deficiencies, especially lack of iron.

VINCENT'S INFECTION—A WARTIME DISEASE: H. T. Dean and D. E. Singleton, Am. Jr. Pub. Health, vol. 35, No. 5, May, '45, p. 433.

The characteristics of the disease are given as pain, necrosis, formation of a pseudomembrane, salivation and a fetid odor. Positive microscopic findings in the absence of these are without diagnostic significance.

The epidemiology of this disease has never been satisfactorily established. This study is based on a survey of the records of the U. S. Public Health Service Dental Clinic at Sheepshead Bay, N. Y., serving training centers for both the Coast Guard and the Maritime Training Service. The annual admission rate for the Coast Guard was 73.1 per 1,000 strength and for the maritime Service, 94.9 per 1,000 strength. Only a few of these were throat infections.

VINCENT'S INFECTION—A WARTIME DISEASE. Observations on the Oral Spirochetal Flora Present in Vincent's Infection: E. G. Hampp, Am. Jr. Pub. Health, vol. 35, No. 5, May, '45, p. 441.

Conclusive evidence has never been presented to show that the spirochetes and fusiform bacilli actually cause these conditions but spirochetes are found deep in the submucosa, in advance of the fusiform bacilli and of necrosis.

A culture method is described using a modified Huntoon's hormone agar in 25 ml. beakers, filled to a depth of approximately 17 mm. After solidification, using a glass pipette with a bore of 4-5 mm., a well approximately 12 mm. deep was made in the center. Scrapings from lesions of Vincent's infections were mixed with a few drops of liquid medium. Some of this was examined by dark-field and a few drops were also placed in the well of the culture medium. Incubation was upright in jars of hydrogen. Spirochetal growth appeared as a white haze extending out from the well. The spirochetes penetrate the agar while the contaminating organisms remain within the well. Incubation periods varied from 36 hours to five days. Using a capillary pipette, subcultures can be taken from the edges of the haze. A high percentage of pure cultures can be obtained this way.

Use of the dark-field facillitates differentiation between the various oral spirochetes. Plates comparing dark-field and stained prep-

arations of Borrelia buccale and Borrelia vincentii as well as the significant characteristics of both are presented. In general the drying and fixing necessary in the preparation of stained smears, shrinks the organism and permits it to flatten out, thus losing its distinguishing spirals.

VINCENT'S INFECTION—A WARTIME DISEASE. Reporting of Vincent's Infection by State Health Departments: C. L. Sebelius, Am. Jr. Pub. Health, vol. 35, No. 5, May, '45, p. 451.

Questionnaires sent out showed that Vincent's infection is reportable in 22 states but there was considerable disagreement on what constitutes Vincent's infection. Reporting was in general very poorly carried out with only a few persons reporting most of the cases. In some states physicians reported the disease as Vincent's infection while dentists reported as gingivitis.

BACILLARY DYSENTERY IN CURACAO, NETHERLANDS, WEST INDIES: A. W. Pot, Am. Jr. Diges. Dis., vol. 12, No. 3, Mar., '45, p. 70.

This survey is based on 404 patients. Endo's medium was used at first but it was found that Bacto S S agar produced a greater number of positives. Cases were either mild or moderately severe. Often there was clinical recovery before the examination was finished.

Agglutination tests were carried out on the isolated strains. Epidemic outbreaks occurred after rainfall. Sanitary conditions in this area are extremely primitive and inadequate.

Nearly all types of the Flexner strain of Shigella paradysenteriae were found with type "W" most frequent. The Shiga bacillus, or Shigella dysenteriae was not found.

PROLIFERATIVE LESIONS IN MULTIPLE MYELOMA WITH SPECIAL REFERENCE TO THOSE OF THE SPLEEN. THE ORIGIN OF THE PLASMA CELL: E. Lowenhaupt, Am. Jr. Path., Vol. 21, No. 1, Jan., '45, p. 171.

These observations are based on 12 autopsied cases of multiple myeloma. It appears to be concerned with the formation of antibody globulins. Evidence is presented to show that plasma cells arise from tissue histiocytes rather than from lymphoid cells.

INITIAL AEROBIC FLORA OF NEWBORN INFANTS: J. C. Torrey and M. K. Reese, Am. Jr. Dis. of Children, Vol. 69, No. 4, April, '45, p. 208.

This study is based on cultures of the throats and noses of 105 full-term infants. In cultures taken within 4 hours of birth 95% showed no growth. Cultures taken from infants 13-18 hrs. old showed no growth in 31% of the nose cultures but in only 4% of the throat cultures. By the end of the first 24 hours no more sterile cultures were obtained either from the nose or throat.

The first organisms found were white staphylococci, alpha or gamma streptococci and an occasional diphtheroid. The noses of infants were more favorable for the coagulase-positive S. aureus than those of adults. Recovery was 9.5% for the first day, 50% the second day and 88.8% between the fourth and eighth days. Pneumococci, beta hemolytic streptococci, H. influenzae, etc., rarely occurred during the first two weeks.

HELP WANTED

Registered Medical Technologist for Berea College Hospital, a small hospital approved by American College of Surgeons. Write Francis S. Hutchins, President.

NEWS AND ANNOUNCEMENTS

CONVENTION NEWS

Annual Convention
A. S. M. T.
San Francisco, Calif.

NOTE CHANGE OF DATES—JUNE 25, 26, 27

Headquarters: Palace Hotel, San Francisco.

Rooms $6.00 up (double). No single.

All reservations should be made through Convention Bureau, Civic Auditorium, San Francisco, Calif. *IMMEDIATELY.*

Chairman of the Nominating Committee has a new address: Mrs. Clara Kruse, M.T. (ASCP), 725 Wilkinson St., Red Wing, Minnesota.

COMMITTEE ON BY-LAWS

It is the purpose of the committee composed of Mary Eichman, Lucille Wallace, Lawrence Ray, Dorothy Meyer and Henrietta Lyle, to submit to the House of Delegates at its next meeting a revised and more concise form of the Articles of Incorporation. It is the intention of the committee to so amend the by-laws that they will include all subject matter that will be deleted from the Articles of Incorporation as well as amending the by-laws that the organization will be in principle an association of state societies.

Mrs. Kathleen C. Bradley, 1340 West Hunting Park Drive, Philadelphia 40, Penna., Chairman of the Program Committee, wishes to announce that the deadline for submission of papers to be eligible for awards is May 15.

We are in need of September, 1945, copies of the *Journal*. Please write the Executive Secretary, Miss Tate, Lafayette, La., if you have a copy you wish to send in.

Minnesota

On February 10, 1946, at St. Joseph's Hospital in Mankato, Minnesota, the Southern Minnesota Society of Medical Technologists was organized. Its slate of officers were elected as follows: Vivian Wolff, New Ulm, president; Arnold Fangmann, Redwood Falls, vice-president; Janet Prevery, Mankato, secretary-treasurer; Esther Wilbrecht, New Ulm, president-elect; Elizabeth Maclay, Rochester board member; Sister Amalia Klopsteg, Faribault board member; Aubrey Lewis, Mankato, editor.

This makes four out of the five prospective districts organized, the fifth, that of The Northern Minnesota Society of Medical Technologists is scheduled to be organized in April at Crookston, Minnesota.

The oldest district society in the state of Minnesota is the Twin Cities Society of which Miss Dorothy Magaw of St. Paul is president; and the second oldest that of The Arrowhead Society of Medical Technologists of which Miss Elizabeth Hudberg of Duluth is president.

Miss Claussen in a letter addressed to the state members not in the national society has this to say:

"What's wrong with this picture? You are a member of the great and active Minnesota Society of Medical Technologists. You have paid your dues, you receive your publication, you attend the meetings when you are able. You know that it is the thing for an up-and-coming M.T. to do.

"You make your living by the profession of Medical Technology. You are interested in this fascinating work. You spent years and hundreds of dollars preparing for the time when you could become 'registered.'

"And yet, there is something wrong. For . . . YOU have not availed yourself of the opportunity of joining the AMST, the great national organization, that is the backbone of the whole medical technology profession.

"Something else is wrong. We have (less than half of the state members belonging) to the national society. Why is this true? . . . Surely it cannot be the few dollars dues. Are not these simply a 'necessary expense,' just as much as the uniform you wear? Or is it that you have 'just put it off,' just never got around to joining?

"I am here to find the answer and to ask you to give the matter your immediate attention. John D. Rockefeller, in his famous creed said, 'I believe that every *right* implies a *responsibility*, every *opportunity* an *obligation*, every *possession* a *duty*.' To me these are inspiring words. To me the *right* to practice the profession of Medical Technology implies the responsibility of doing all in my power to make a good job of it. The opportunity of joining this great ASMT implies an obligation to join; the possession of the initials M.T. (ASCP) carries with it the duty of seeing to it that they really mean something, not only when we first earn them but on through the years."

Wisconsin

The Wisconsin Association of Medical Technologists held its annual convention Saturday, October 13, and Sunday, October 14. The Saturday afternoon sessions met at the Plankinton House. One hundred and three medical technologists, an increase of eleven per cent over last year, were present at the scientific program.

Dr. Chester W. Kurtz, professor of medicine at the University of Wisconsin, gave an account of the State's plan for reclaiming young victims of rheumatic fever. Dr. Armand Quick, professor of biochemistry at Marquette University, again presented in his lucid manner the theory and application of prothrombin time determination. Dr. Israel Davidsohn, editor of Technical Bulletin of the Registry of Medical Technologists, informed the audience on recent developments of Rh factor sensitivity tests.

At the traditional problem clinic, bacteriologist Mary Jane Jessel, biochemist Margaret Perry of Columbia Hospital and hematologist Estelle Downer of Milwaukee County Hospital were on hand to give expert technical advice to those requesting it.

Sixty-eight members and guests attended the dinner to hear Dr. Max Fox, director of Southview Hospital relate his experiences in

his inimitable fashion. The Plankinton House served a delicious turkey dinner.

Next morning (Sunday) a group of fifty-five technologists partook of a very satisfying breakfast at St. Joseph's Hospital. Afterwards we had the pleasure of listening to the following: (1) Chest surgeon, Dr. John D. Steele, whose talk on lung abscesses was a model of clarity and completeness and (2) a young Milwaukee specialist in internal medicine, Dr. Nathan Grossman, who developed reasons supporting the idea that rheumatic fever and allergy are related. This concluded the scientific sessions.

The nominating committee announced the following members elected to office for 1945-46: Geraldine Miller, president-elect; Sylvia Anderson, secretary; Esther Lemont, treasurer; Sister M. Benonia Reiser, director for 4th district.

Meetings expected for the coming year are: (1) May 1-2-3, 1946. Chicago at Tri-State Hospital Assembly. Palmer House. (2) October, 1946. Madison. Annual convention.

YOUNG PEOPLE ARE CROWDING THE COLLEGES!

Recruiting students for Medical Technology is as important as ever. Now is the time to visit the counselors at your local high schools and nearby colleges and universities. Tell them of the need for well trained Medical Technologists. Ask permission to participate in their Vocational Conferences. Give them a booklet of information and a list of approved schools of Medical Technology so they can help their students meet the Registry requirements.

For additional material or information write to:

REGISTRY OF MEDICAL TECHNOLOGISTS
of the
AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS
Ball Memorial Hospital
Muncie, Indiana